AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

LETTER TO THE EDITOR

Clostridium scindens Is Present in the Gut Microbiota during *Clostridium difficile* Infection: a Metagenomic and Culturomic Analysis

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KEYWORDS *Clostridium difficile*

Clostridium scindens was first isolated in 1984 during work on the gut microbiota's steroid production (1). It became of great interest in 2015 with the study proposed by Buffie et al. (2). The authors demonstrated that *C. scindens* was associated with resistance to *Clostridium difficile*. *C. scindens*, thanks to its 7-alpha-dehydroxylase enzyme, transforms primary bile acids into secondary bile acids, which are known to inhibit *C. difficile* germination and growth (2). After this demonstration, *C. scindens* was proposed as a probiotic against *C. difficile*, and relevant patents were filed with the aim of testing the effectiveness of *C. scindens* (3). Since that major study, despite overenthusiasm that extended even beyond the scientific community (4), there has been, to the best of our knowledge, no trial report of any use of *C. scindens* in human.

This study was approved by the ethics comity of the IHU Méditerranée Infection under number 2016-011.

With the objective of studying the gut microbiota in relation to *C. difficile* infections (CDI), we analyzed 30 stool samples from patients with CDI by metagenomics. These stool samples, collected from 30 patients suffering from diarrhea, were all positive for B toxin by real-time PCR (tested with Xpert *C. difficile* [Cepheid, Sunnyvale, CA, USA]). The mean PCR cycle threshold (C_T) value for B toxin was 26.7 (information was available for 24/30 samples), which represents good clinical relevance for CDI diagnosis as described elsewhere (5). We also analyzed 27 stool samples from healthy adults, which were found to give negative results for *C. difficile* by reverse transcription-PCR (RT-PCR). Sequencing was performed on the V3-V4 region of the 16S gene by the use of MiSeq technology (Illumina, San Diego, CA, USA) as previously described (6). We obtained means of 108,924 and 213,245 reads per sample for CDI and stool samples from healthy adults, respectively. Operational taxonomic units (OTUs) corresponding to *C. scindens* were detected in 13/30 (43.3%) and 4/27 (21%) of cases for CDI and stool samples from healthy adults, respectively. *C. scindens* was significantly overrepresented among the members of the CDI group ($P = 0.0004$ [Fisher's exact test]). In the CDI group, *C. scindens* OTUs accounted for 0.058% and *C. difficile* OTUs accounted for 0.219% of the mean relative abundances for each sample. In the control group, *C. scindens* OTUs accounted for 0.011% of the mean relative abundance for each sample and no OTU attributed to *C. difficile* was detected.

In parallel, as a part of a large-scale culturomic study (6), we analyzed six fresh stool samples from CDI patients. We used six different culture conditions: growth in aerobic and anaerobic blood bottles with adjunction of rumen and/or sheep blood, incubation at 37°C, and seeding on 5% sheep blood agar. Before inoculation into blood bottles was performed, stool samples were exposed to ethanol for 4 h in order to facilitate sporulation (7). Surprisingly, in 1 of the 6 stool samples (taken from a 90-year-old man, treated for a severe recurrent episode of CDI), we cocultivated *C. scindens* and *C. difficile*.

Accepted manuscript posted online 28 February 2018

Citation Amrane S, Bachar D, Lagier JC, Raoult D. 2018. *Clostridium scindens* is present in the gut microbiota during *Clostridium difficile* infection: a metagenomic and culturomic analysis. J Clin Microbiol 56:e01663-17. https://doi.org/10.1128/JCM.01663-17.

Editor Andrew B. Onderdonk, Brigham and Women's Hospital

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TABLE 1 Characteristics of the patients with codetection of *C. scindens* and *C. difficile* by culture

Sample	Sex[a]	Age	Country	Clinical condition	Culturomic protocol (reference no.)	No. of species detected
Amazonia 1	F	10 yrs	French Guyana	Healthy	70 conditions (6)	137
Amazonia 2	F	16 yrs	French Guyana	Healthy	70 conditions (6)	131
Kwashiorkor 10	M	2 mo	Senegal	Malnutrition, kwashiorkor	18 conditions (6)	117
Raiatea	M	39 yrs	French Polynesia	Healthy	70 conditions (6)	136
CDI + OH 4	M	90 yrs	France	Recurrence of CDI	Stool sample preincubated with ethanol; 6 conditions	24

[a]F, female; M, male.

The two bacterial species were identified by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) (Microflex; Bruker, Billerica, MA, USA) with a score indicating good species identification (6).

To the best of our knowledge, there is no publication reporting the cooccurrence of *C. difficile* and *C. scindens* in the same stool sample. In our laboratory, since 2012, we have subjected 55 stool samples to culturomic analysis under comparable culture conditions (6). We detected a cooccurrence of *C. scindens* and *C. difficile* in 4 (7.2%) (Table 1).

Among the published bacteriotherapies tested against CDI (8, 9), none included *C. scindens*. As we detected *C. scindens* and *C. difficile* in the same stool samples, we can suggest that *C. scindens* does not have the power to inhibit the growth of *C. difficile* on its own.

ACKNOWLEDGMENTS

We thank culturomic students for their work and Magdalen Lardière for her assistance in English language editing.

This work has benefited from French Republic support, managed by the Agence Nationale pour la Recherche, including the Programme d'Investissement d'avenir (reference Méditerranée Infection 10-IAHU-03).

We declare that we have no conflicts of interest.

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